The Guinea Pig as a Model for Isoniazid-induced Reactions

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Summary | Daily injections of isoniazid were given to evaluate the guinea pig as a model in the study of such human side effects as coagulopathy syndrome, pyridoxine deficiency and hepatic lesions. Mild hepatitis was demonstrated by increases in serum glutamic oxaloacetic transaminase and sorbitol dehydrogenase levels. Hepatic lesions ranged from focal areas of inflammatory cell infiltrate to necrosis. Cutaneous testing demonstrated that the hepatitis probably was a hypersensitivity reaction to both isoniazid and isonicotinic acid. Eosinophilia and eosinophils present in hepatic lesions also suggested a hypersensitivity reaction.

Increases in prothrombin time, partial thromboplastin time and fibrin split products as well as decreases in fibrinogen and thrombocytes indicated that a coagulopathy syndrome had been produced. Specifically, a disseminated intravascular coagulation-like syndrome occurred in treated animals. Isoniazid treatment also induced a pyridoxine deficiency which was demonstrated by decreases in serum pyridoxine concentrations. This deficiency was associated with demyelination of the sciatic nerve. Because the abnormalities found in these guinea pigs were similar to those reported in man, this species appears to be a good model for studying isoniazid-induced hypersensitivity hepatitis and its possible linkage to the syndrome of disseminated intravascular coagulation.

Key Words | Isoniazid — Hepatitis — Hypersensitivity — Guinea pig

Isoniazid is considered by many to be the most effective drug in the control and treatment of tuberculosis (1); however, it does have the potential of producing some serious side effects. The most prominent of these in man includes decreased serum pyridoxine concentrations (2), hepatic toxicity (3-5) and possibly a coagulopathy syndrome (6).

Decreased serum pyridoxine values occur rather frequently in human patients receiving isoniazid and are probably due to the drug's competition with pyridoxal phosphate for the enzyme apotryptophanase (7). One of the signs of a pyridoxine deficiency in man is peripheral neuropathy which, initially, is sensory and later may progress to motor involvement (8). Histologically, isoniazid-induced peripheral neuropathy in rats is characterized by fragmentation of the myelin sheath of the small and medium sized sensory and mixed nerve fibers to the muscle spindles (9). In addition, there is concomitant remyelination and collateral sprouting of motor axons. Numerous studies on humans and rats have shown that isoniazid-induced peripheral neuropathy can be prevented or reversed by the administration of pyridoxine (2,10).

The frequency of isoniazid-induced liver disease is highly variable in humans,

ranging from 8.7 to 22.9% (3,5). Changes in liver function tests have been demonstrated by increased alkaline phosphatase activity, marked elevation of serum glutamic oxaloacetic transaminase activities and increases in serum glutamic pyruvic transaminase in symptomatic as well as asymptomatic hepatitis patients (11).

The cause of isoniazid-induced hepatic injury is uncertain, but it is believed that its pathogenesis represents a hypersensitivity reaction to isoniazid or its metabolites (4,12-18). Presumably, the drug attaches to a liver cell protein in such a way as to make it immunogenic (19).

A secondary effect of isoniazid-induced hepatic damage may be a coagulopathy syndrome. Approximately 85% of the patients with liver disease of various kinds have at least one abnormality in tests of clotting function (20). Disseminated intravascular coagulation has been described in various types of liver disease, including isoniazid-induced liver damage (6).

The purpose of this study was to evaluate isoniazid toxicity in guinea pigs and determine if the effects were similar to those which occurred in man.

Materials and Methods

Animals: One hundred fifty female Dla:(DH) guinea pigs[6] *(Cavia porcellus)* weighing 300-500 g were randomly assigned into five groups of 30 guinea pigs each. Each group was given twice-daily intramuscular injections in the left rear leg of isoniazid[7] in divided doses for 11 weeks. Groups 1-4 received isoniazid at doses of 10, 30, 60 and 100 mg/kg body weight/day respectively; Group 5 served as untreated controls and received 2 ml sterile saline per day administered in the same manner. The guinea pigs were weighed weekly and the quantity of isoniazid was adjusted according to the weight gain or loss.

Evaluation of hypersensitivity reaction: The guinea pigs were tested for a cutaneous delayed-type hypersensitivity

reaction to isoniazid[8] and its metabolite, isonicotinic acid[8] (21). One week after the start of treatment, all guinea pigs were immunized with prepared conjugates of isoniazid and isonicotinic acid to human serum albumin[8] and guinea pig serum albumin[8] (22). Ten milligrams of each of these conjugates in Freund's complete adjuvant[9] were injected intradermally in the flank of each guinea pig. The guinea pigs were reimmunized at weeks 4 and 7.

Cutaneous sensitivity test was measured on the abdomen of each guinea pig 72 hours before the animal was killed. Each guinea pig was tested intradermally with 10.0 mg of each of the following soluble antigens in a saline solution: isoniazid-human albumin, isonicotinic acid-human albumin, isoniazid-guinea pig albumin, isonicotinic acid-guinea pig albumin, isoniazid, isonicotinic acid, human albumin, guinea pig albumin and tuberculin.[10] At the time of death, the injection sites were examined for erythema and edema or necrosis. The cutaneous lesions were scored on a range of 0-7, depending on the type of reaction and the antigen which caused the reaction.

Hematologic determinations: Five guinea pigs from each group were exsanguinated on weeks 1, 3, 5, 7, 9 and 11 of the study by cardiac puncture following anesthesia with 1% halothane.[11] Blood collected in ethylenediamine tetracetic acid tubes was used to determine the thrombocyte count by a manual method[12] and an automated counter.[13] The presence or absence of eosinophilia was determined by a complete blood count. The plasma obtained from blood collected in sodium citrate tubes was used to assay for fibrinogin, prothrombin time and activated partial thromboplastin time (23-25). Blood collected in the fibrin split products tubes was used to detect the presence of fibrin split products utilizing the hemagglutination inhibition immunoassay test (26). Pyridoxine was assayed spectrophoto-fluorometrically[14] (27). The serum obtained from the blood collected in dry tubes was used to assay for serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase and sorbitol dehydrogenase.

Histopathology: After collection of the blood samples, a complete necropsy was done on each guinea pig. Tissue samples from the heart, kidney, liver, brain and sciatic nerve from the right rear leg were fixed in neutral buffered 10% formalin. Paraffin imbedded

sections 5 μm thick were made from each tissue and stained with hematoxylin and eosin. In addition, sections of the sciatic nerve were stained with Luxol's fast blue to demonstrate myelin, and sections of the liver were post-fixed in 1% osmium tetroxide to demonstrate fatty changes. The histopathological evaluation of the liver and nerve were scored on ranges of 0-6 and 0-5, respectively, depending on the severity of the lesions, with the highest number representing the most severe lesion.

Statistical analysis: A two-way analysis of variance was done to determine if an overall significance ($p < 0.05$) existed between the guinea pig groups for fibrinogen, activated partial thromboplastin time, prothrombin time, serum glutamic pyruvic transaminase, serum glutamic oxaloacetic transaminase, sorbitol dehydrogenase, pyridoxine, eosinophil count and thrombocyte count at each sampling period and between the weeks of the study. Next, a one-way analysis of variance was done on each parameter for each week of the study to determine if a significant difference ($p < 0.05$) existed between the groups. The least-squares difference test was used to compare each treated group mean to the control group mean for each of the six weeks of the project. The mean score values for fibrin split products, skin sensitivity and nerve and

liver histopathology were analyzed nonparametrically using the Kruskal Wallis test. The presence of a disseminated intravascular coagulation-like syndrom was calculated using Fisher's Exact test. A value of $p < 0.05$ was considered statistically significant.

Results

The animals appeared to be in good health throughout the treatment period. However, the rate of weight gain was depressed in the treated groups when compared to the controls. The food and water consumption for all groups appeared to be similar. No attempt was made, however, to measure each group's consumption of food and water.

Evaluation of hepatic damage: Serum glutamic pyruvic transaminase activities were similar in the control and isoniazid-treated guinea pigs throughout the experimental period. Sorbitol dehydrogenase and serum glutamic oxaloacetic transaminase values of the isoniazid-treated guinea pigs were increased beginning in the third week when compared to the controls (Tables 1 and 2, respectively). Significant increases ($p < 0.05$) in the sorbitol dehydrogenase values of the isoniazid-treated guinea pigs occurred throughout the project except in the fifth and seventh weeks when the control sorbitol dehydrogenase

Table 1

Comparison of the mean sorbitol dehydrogenase levels between control and isoniazid-treated guinea pigs

| Treatment - | Sorbital dehydrogenase levels by weeks of treatment (Sigma units/ml) | | | | | |
	1	3	5	7	9	11
Control	76.9 ± 53.7[a]	72.4 ± 19.5	132.6 ± 21.5	139.2 ± 12.5	66.4 ± 13.2	121.8 ± 20.9
10 mg/kg	132.8 ± 50.7	102.8 ± 37.7	167.9 ± 80.5	178.1 ± 20.1[b]	128.1 ± 75.4	209.6 ± 54.1[b]
30 mg/kg	25.1 ± 12.5	200.2 ± 30.9[b]	151.0 ± 74.7	174.3 ± 36.4[b]	156.2 ± 24.9[b]	183.5 ± 7.5[b]
60 mg/kg	131.6 ± 60.6	188.9 ± 65.5[b]	193.3 ± 32.8	178.4 ± 11.8[b]	241.3 ± 27.2[b]	181.5 ± 71.7
100 mg/kg	98.7 ± 39.6	241.2 ± 46.5[b]	201.8 ± 42.6	167.8 ± 19.4[b]	250.9 ± 44.9[b]	185.0 ± 26.7[b]

[a]Mean ± standard deviation of values obtained from five guinea pigs
[b]$p < 0.05$ compared to the control group

Table 2

Comparison of the mean serum glutamic oxaloacetic transaminase levels between control and isoniazid-treated guinea pigs

| Treatment | Serum glutamic oxaloacetic transaminase levels by weeks of treatment (Reitman-Frankel units/ml) | | | | | |
	1	3	5	7	9	11
Control	91.5 ± 33.9[a]	66.4 ± 18.1	80.8 ± 12.8	61.5 ± 17.5	89.2 ± 28.3	69.4 ± 21.2
10 mg/kg	159.5 ± 96.2	118.2 ± 39.6	75.0 ± 14.2	243.0 ± 21.9[b]	93.0 ± 30.3	83.6 ± 51.8
30 mg/kg	174.0 ± 9.8	53.5 ± 12.2	83.6 ± 28.4	72.0 ± 6.9	191.5 ± 25.6[b]	155.0 ± 25.6[b]
60 mg/kg	138.5 ± 96.8	56.7 ± 16.7	152.5 ± 27.4[b]	87.5 ± 39.6	157.2 ± 45.5[b]	116.0 ± 31.5[b]
100 mg/kg	156.4 ± 53.4	146.0 ± 66.6[b]	73.4 ± 25.1	423.5 ± 256.6[b]	176.4 ± 45.8[b]	155.6 ± 17.8[b]

[a]Mean ± standard deviation of values obtained from five guinea pigs
[b]$p < 0.05$ compared to the control group

values also were elevated (Table 1). The results shown in Table 1 indicate that the three higher dose groups developed early liver damage which continued to the end of the study. The most severe liver damage occurred in the ninth week in the 60 and 100 mg/kg groups when the mean activity values were 241.3 and 250.9 Sigma units/ml, respectively.

A significant increase (p<0.001) in the serum glutamic oxaloacetic transaminase activity was first demonstrated in the third week in the 100 mg/kg group and in the fifth week in the 60 mg/kg group (Table 2). By the ninth week, significant increases (p<0.05) in the serum glutamic oxaloacetic transaminase activities were found in the 30, 60 and 100 mg/kg groups, and this elevation persisted in the eleventh week.

Evaluation of hypersensitivity reaction: The control guinea pigs had a slight eosinophilia at the beginning of the study. By the third week, however, all the isoniazid-treated groups had significantly elevated (p < 0.05) eosinophil counts which continued through the eleventh week. From the third week, the eosinophil counts for the control group and the isoniazid-treated groups ranged from 45.6 to 125.4/mm³ and 152.2 to 586.1/mm³, respectively No other abnormalities were found in the complete blood counts.

Evaluation of a disseminated intravascular coagulation syndrome: Fibrinogen concentrations of isoniazid-treated guinea pigs were significantly decreased (p<0.001) each week at every dose when compared to the controls (Table 3). The only exception to this was that the 10 mg/kg group was not significantly decreased in the fifth week.

Fibrinogen concentrations decreased for all treatment groups by the end of the first week of the study. The guinea pigs treated with the lowest dosage (10 mg/kg) generally had higher values of fibrinogen than the other treatment groups until the seventh week of the study, when these were not significantly higher (p<0.05) than those of the other isoniazid-treated groups.

The prothrombin times for the isoniazid-treated guinea pigs were significantly increased (p < 0.05) each week at every dose when compared with the control group values (Table 4). In addition, the 100 mg/kg group had longer prothrombin times than the other treated groups; these were significantly longer (p<0.05) at the seventh and eleventh weeks.

The activated partial thromboplastin times for the isoniazid-treated guinea pigs were consistently prolonged when compared with the control guinea pigs. However, the

Table 3

Comparison of the mean fibrinogen levels between control and isoniazid-treated guinea pigs

Treatment	Fibrinogen levels by weeks of treatment (mg/dl)					
	1	3	5	7	9	11
Control	192.6 ± 39.9ᵃ	232.0 ± 14.4	153.8 ± 36.4	270.8 ± 87.6	274.2 ± 95.6	363.2 ± 29.6
10 mg/kg	96.8 ± 34.8ᵇ	103.5 ± 1.9ᵇ	154.2 ± 41.4	95.4 ± 11.7ᵇ	96.4 ± 2.2ᵇ	111.0 ± 31.9ᵇ
30 mg/kg	44.0 ± 0.7ᵇ	48.2 ± 2.0ᵇ	59.0 ± 10.3ᵇ	54.6 ± 8.9ᵇ	62.1 ± 0.8ᵇ	86.6 ± 8.8ᵇ
60 mg/kg	39.0 ± 3.2ᵇ	39.6 ± 1.5ᵇ	41.0 ± 2.2ᵇ	43.0 ± 1.1ᵇ	39.7 ± 2.1ᵇ	32.7 ± 23.4ᵇ
100 mg/kg	40.6 ± 1.1ᵇ	39.4 ± 1.9ᵇ	47.8 ± 21.7ᵇ	40.4 ± 2.2ᵇ	35.8 ± 4.1ᵇ	5.25 ± 7.9ᵇ

ᵃMean ± standard deviation of values obtained from five guinea pigs
ᵇp<0.001 compared to the control group

Table 4

Comparison of the mean prothrombin time between control and isoniazid-treated guinea pigs

Treatment	Prothrombin time by weeks of treatment (seconds)					
	1	3	5	7	9	11
Control	15.6 ± 4.0ᵃ	13.2 ± 1.9	16.6 ± 3.0	15.9 ± 3.1	17.6 ± 3.5	16.3 ± 5.3
10 mg/kg	28.2 ± 8.8ᵇ	36.5 ± 3.8ᵇ	26.9 ± 0.9ᵇ	31.4 ± 7.1ᵇ	35.8 ± 3.9ᵇ	30.2 ± 2.7ᵇ
30 mg/kg	24.9 ± 2.1ᵇ	35.5 ± 17.4ᵇ	32.9 ± 6.8ᵇ	33.7 ± 5.4ᵇ	33.3 ± 2.1ᵇ	33.7 ± 3.8ᵇ
60 mg/kg	32.4 ± 0.7ᵇ	45.4 ± 15.5ᵇ	34.9 ± 9.0ᵇ	38.6 ± 4.1ᵇ	35.8 ± 6.9ᵇ	34.1 ± 3.9ᵇ
100 mg/kg	29.6 ± 4.3ᵇ	38.5 ± 6.5ᵇ	32.8 ± 2.0ᵇ	55.6 ± 12.2ᵇ	39.5 ± 9.6ᵇ	42.3 ± 4.9ᵇ

ᵃMean ± standard deviation of values obtained from five guinea pigs
ᵇp<0.05 compared to the control group

results were variable and only the higher iso- niazid doses, 30, 60 and 100 mg/kg, produced significantly prolonged times (p<0.05) in half or more of the sampling times (Table 5).

Fibrin split products were not pre- sent in any guinea pig in any group through the first 7 weeks of the study. By the ninth week, however, four guinea pigs in the 30 mg/kg group and two each in the 60 and 100 mg/kg groups had fibrin split products which con- tinued to be detectable in these groups through the eleventh week. The 60 and 100 mg/kg groups had four guinea pigs each with fibrin split products, and the 30 mg/kg group had one.

The thrombocyte count did not vary significantly between the isoniazid-treated guinea pigs and the control guinea pigs through the first 7 weeks of treatment. The 60 and 100 mg/kg groups had significantly de- creased thrombocyte counts in the ninth week (p<0.01) and eleventh week (p<0.05) as com- pared to the controls. The mean thrombocyte counts in the ninth week in the 60 and 100 mg/kg groups were 233,000 and 272,000, re- spectively, compared with a control mean of 614,000. In the eleventh week, the mean thrombocyte counts were 252,000, 373,000, and 464,000 for the 60 mg/kg, the 100 mg/kg and the control groups, respectively.

The criteria for a disseminated intra- vascular coagulation-like syndrome, including relative thrombocytopenia, presence of fibrin split products, decreased fibrinogen, increased prothrombin time and increased activated par- tial thromboplastin time were met in indi- vidual guinea pigs in isoniazid-treated groups beginning in the seventh week. Evidence of a disseminated intravascular coagulation-like syndrome was found in four of the five pigs in the 60 mg/kg group in both the ninth and eleventh weeks and in four of the five pigs in the 100 mg/kg group in the eleventh week.

Evaluation of pyridoxine deficiency: Pyridoxine concentrations of the isoniazid- treated guinea pigs were consistently lower than the controls throughout the experimental period (Table 6). Each isoniazid group had sig- nificantly decreased (p<0.05) pyridoxine con- centrations in half or more of the testing periods. The 100 mg/kg group had significantly decreased (p<0.05) pyridoxine concentrations each week except the ninth week.

Evaluation of hypersensitivity reac- tions: The control guinea pigs developed a reac- tion to the tuberculin when first tested at the third week. This reaction consisted of edema, inflammation and necrosis at the site of injec- tion. None of these guinea pigs demonstrated

Table 5

Comparison of the mean activated partial thromboplastin time between control and isoniazied-treated guinea pigs

Treatment	Activated partial thromboplastin time by weeks of treatment (seconds)					
	1	3	5	7	9	11
Control	28.6 ± 4.6[a]	33.8 ± 2.7	31.0 ± 2.9	32.7 ± 1.6	29.1 ± 5.1	37.8 ± 2.2
10 mg/kg	42.9 ± 3.2[b]	38.7 ± 9.9	38.5 ± 9.8	44.5 ± 6.7	30.1 ± 2.4	40.2 ± 6.0
30 mg/kg	49.4 ± 3.2[b]	29.5 ± 2.1	49.4 ± 9.8[b]	50.7 ± 3.1	46.6 ± 3.2[b]	45.1 ± 5.4
60 mg/kg	49.6 ± 2.3[b]	52.1 ± 7.9[b]	35.0 ± 9.0	43.8 ± 1.9	47.0 ± 3.4[b]	59.1 ± 26.0[b]
100 mg/kg	47.0 ± 6.3[b]	49.2 ± 7.5[b]	34.6 ± 4.5	109.4 ± 54.2[b]	54.3 ± 13.5[b]	48.9 ± 8.3[b]

[a]Mean ± standard deviation of values obtained from five guinea pigs
[b]p<0.05 compared to the control group

Table 6

A comparison of the mean pyridoxine levels between the control and the isoniazid-treated guinea pigs

Treatment	Pyridoxine levels by weeks of treatment (x10⁻⁴ ug/ml)					
	1	3	5	7	9	11
Controls	47.4 ± 7.9[a]	39.3 ± 3.8	36.5 ± 6.1	50.5 ± 7.0	35.7 ± 6.6	38.1 ± 7.7
10 mg/kg	33.3 ± 2.5[b]	33.2 ± 2.4[b]	34.7 ± 3.1	33.3 ± 2.2[b]	33.2 ± 1.4	33.6 ± 1.0
30 mg/kg	37.2 ± 2.9[b]	32.3 ± 1.1[b]	34.1 ± 2.0	36.8 ± 1.8[b]	32.2 ± 0.8[b]	32.4 ± 1.5
60 mg/kg	37.4 ± 4.0[b]	32.1 ± 0.9[b]	30.5 ± 3.4[b]	35.9 ± 2.1[b]	31.5 ± 1.1	33.2 ± 5.1
100 mg/kg	30.5 ± 4.6[b]	32.5 ± 0.7[b]	29.8 ± 1.0[b]	39.4 ± 5.1[b]	32.9 ± 1.4	30.8 ± 1.6[b]

[a]Mean ± standard deviation of values obtained from five guinea pigs
[b]p<0.05 compared to the control group

any reaction to the other agents. By the fifth week, two of the five controls developed an inflammatory reaction to the isoniazid conjugates. The control guinea pigs gradually showed increased reactivity to the conjugates. By the seventh week, one control guinea pig had a necrotic reaction to the isoniazid conjugates. By the eleventh week, two of the five controls had necrotic reactions to both isoniazid and isonicotinic acid conjugates.

Guinea pigs receiving isoniazid at all dosages developed cutaneous reactions to the conjugates when first tested at the third week. When cutaneously tested at the third week, the isoniazid-treated guinea pigs showed more severe reactions to the isoniazid conjugates than to the isonicotinic acid conjugates. At this first cutaneous test, all but one guinea pig developed a necrotic reaction to one or both of the isoniazid conjugates, while only one demonstrated a necrotic response to the isonicotinic acid conjugates, in addition to the isoniazid conjugates. By the ninth week, all but three of the guinea pigs receiving isoniazid developed necrotic reactions to both the isoniazid and isonicotinic acid conjugates. However, the severity of the necrotic reactions to both the isoniazid conjugates diminished somewhat during the ninth and eleventh weeks, while the severity of the necrotic reactions to the isonicotinic acid conjugates reached a peak during those 2 weeks.

The sensitivity that the isoniazid-treated guinea pigs showed for the conjugates of isoniazid and isonicotinic acid generally was more severe than that for tuberculin. The control guinea pigs also demonstrated increased reactivity to the isoniazid and isonicotinic acid conjugates, but not until the seventh, ninth and eleventh weeks.

Pathology: The only gross lesions seen were yellowish-brown discolorations of the livers of some of the guinea pigs in the 60 and 100 mg/kg dosage groups in the ninth and eleventh weeks.

Three control guinea pigs had histologic hepatic lesions; one had a small focal area of hepatic necrosis with no inflammatory cell involvement, while the other two each had a small focal area of necrosis with some neutrophils present. The mean liver evaluation score for the control guinea pigs was 0 each week except the seventh and ninth weeks when it was 0.4 and 1.2, respectively.

The isoniazid-treated guinea pigs had significant hepatic damage when compared with the controls. The hepatic damage occurred primarily in the 30, 60 and 100 mg/kg groups where the mean liver evaluation score ranged from 1.0 in the 30 mg/kg group in the first week to a high of 5.2 in the 100 mg/kg group in the seventh week. A significant amount of hepatic damage was seen in the 10 mg/kg group during the fifth week when the mean liver evaluation score was 2.6. Consistent significant ($p < 0.05$) hepatic damage in the higher isoniazid doses began in the seventh week and continued through the end of the study.

Hepatic changes in the isoniazid-treated guinea pigs included focal aggregation of inflammatory cells (eosinophils and neutrophils), focal areas of necrosis occasionally associated with inflammatory cells and diffuse areas of hepatocyte degeneration and regeneration. Focal and diffuse areas of fatty degeneration among hepatocytes also was present.

None of the control guinea pigs had sciatic nerve lesions. Demyelination of the sciatic nerve was first found in the third week in two isoniazid-treated guinea pigs, one each in the 60 and 100 mg/kg groups. The incidence of demyelination continued to be low in the fifth week, occurring in only two animals in the 60 mg/kg group. In the seventh week, three of five guinea pigs in each of the 60 and the 100 mg/kg groups had demyelination of the sciatic nerve. The incidence of demyelination increased in the ninth week involving all guinea pigs at the higher dosages as well as some guinea pigs in the 10 and 30 mg/kg groups. A similar incidence was found in the eleventh week, except that no lesions were found in the 10 mg/kg group. The occurrence of nerve lesions was statistically significant ($p < 0.05$) in the 60 mg/kg group in the ninth week and in the 100 mg/kg group ($p < 0.01$) in the ninth and eleventh weeks.

The nerve lesions in the isoniazid-treated guinea pigs became progressively more severe as the treatment continued and appeared to be dose-related. Infiltration of the sciatic nerve with inflammatory cells was characterized by eosinophils, neutrophils and macrophages. Soon after the appearance of the inflammatory cells, small eosinophilic granules were formed among the fibers and in vacuole-like spaces. Other abnormalities seen during

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this time, or shortly thereafter, included swelling of the myelin sheaths adjacent to the groups of inflammatory cells and intimal proliferation of nearby arteries. These legions progressed to large areas containing many inflammatory cells and few or no axons in the 60 and 100 mg/kg groups.

Discussion

The experimental injection of guinea pigs with isoniazid appeared to produce laboratory and pathologic changes similar to those reported for isoniazid-induced hepatic damage in humans (19,26,28). There were increases in the serum glutamic oxaloacetic transaminase and sorbitol dehydrogenase activities as well as differences in liver lesions between isoniazid-treated guinea pigs and controls. The delayed hypersensitivity demonstrated in this study tends to indicate an allergic rather than a toxic reaction to the drug. The study also provides a possible linkage between isoniazid-induced liver damage and a syndrome resembling disseminated intravascular coagulation as well as a parallel pyridoxine deficiency.

Elevated sorbitol dehydrogenase and moderate increase in serum glutamic oxaloacetic transaminase activities for the isoniazid-treated guinea pigs suggested that while focal liver damage did occur, the damage was not massive and involved only a portion of the hepatic tissue. These clinical laboratory findings were substantiated by microscopic lesions of mild hepatitis in the isoniazid-treated guinea pigs. The areas of inflammatory cell infiltrate, with or without necrosis, were focal and involved only a small portion of the liver. These histologic changes in the liver were not indicative of a toxic reaction but instead tend to indicate that a sensitivity reaction has taken place.

The results of this study demonstrated induction of a specific cutaneous delayed-type hypersensitivity to isoniazid and its metabolite, isonicotinic acid, when they were conjugated to a protein, serum albumin. By demonstrating a hypersensitivity reaction to both isoniazid and isonicotinic acid, it appears that the guinea pig is similar to man (29) and the rhesus monkey (30) in the liver's ability to metabolize a portion of the isoniazid to isonicotinic acid.

The guinea pigs first became sensitized to isoniazid and later to isonicotinic acid.

The hypersensitivity was a specific reaction to isoniazid and isonicotinic acid and not to the protein to which they were conjugated, since no reaction occurred to either the human albumin or to the guinea pig albumin when they were tested separately. All guinea pigs reacted to the tuberculin because they had been sensitized with Freund's complete adjuvant. This reaction was expected and thus was used as an indicator to ensure that the sensitivity test was done properly. Skin test reactions in isoniazid-treated guinea pigs developed sooner and were more intense than in control guinea pigs which indicated that the test animals were more sensitized to isoniazid and isonicotinic acid than the controls. This coincided with the development of hepatic lesions in the isoniazid-treated guinea pigs and would seem to indicate that the liver lesions, like the skin reactions, were a result of hypersensitivity to isoniazid, isonicotinic acid or both.

The occurrence of eosinophilia in the isoniazid-treated guinea pigs beginning in the third week also suggested a hypersensitivity reaction. Eosinophilia is common to antigen-antibody reactions including instances of parasitism and in disease leading to degeneration of body protein (31). The cutaneous sensitivity reaction seen in all guinea pigs could account for some, but not all, of the eosinophil increase. This tends to indicate that a hypersensitivity reaction occurred elsewhere in the isoniazid-treated guinea pigs; the most likely site appeared to be the liver. The presence of necrosis and inflammatory cell infiltrates in hepatic lesions supports the theory that the liver is the site of a hypersensitivity reaction.

The diagnosis of a syndrome resembling disseminated intravascular coagulation in the isoniazid-treated guinea pigs was suggested by the development of a relative thrombocytopenia, decreased fibrinogen, prolonged activated partial thromboplastin time and prothrombin time, and elevated fibrin split products. However, the presence of fibrin split products was inconsistent, as was the decrease in thrombocytes. A possible explanation for the failure to detect fibrin split products earlier and in higher titers is that the test, while sensitive for human fibrin split products, may not be sufficiently sensitive for the guinea pig. The cause of the coagulopathy syndrome demonstrated in this study was probably isoniazid-induced liver damage because the liver is the

primary site for the synthesis of many of the clotting factors. An obvious hemorrhagic disorder, including subcutaneous hemorrhage and microthrombi, was not seen in any of the isoniazid-treated guinea pigs. This might suggest that, while statistically significant, the thrombocytopenia might be a result of wide variations within a normal range resulting in no clinical significance. It is possible, however, that the thrombocytopenia would have progressed and that a hemorrhagic syndrome would have been demonstrated had the study continued.

The isoniazid-induced pyridoxine deficiency in the treated guinea pigs appeared to have produced peripheral nerve lesions similar to those reported in other laboratory animals as well as man (8,9,32). The nerve lesions associated with isoniazid therapy were similar to those described for Wallerian degeneration of the axon (33), with the additional component of inflammatory cells being present. The nerve lesions did not involve the entire nerve. In most of the guinea pigs, the lesions were confined to one or more areas with normal appearing axons and myelin sheaths in the other areas. The lesions did not produce a clinically evident motor deficit.

No attempt was made in this study to reverse the peripheral neuropathy by supplementing the guinea pigs' diet with pyridoxine. A reversal of the neuropathy after pyridoxine administration in the isoniazid-treated guinea pigs would have provided definitive evidence for a linkage between isoniazed administration, pyridoxine deficiency and peripheral neuropathy.

The guinea pig is a valuable potential model in the study of side-effects of isoniazid therapy and may be used to improve evaluation, understanding and prediction of these detrimental reactions.

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